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Research Article

IN VITRO ANTIDIABETIC AND *IN VIVO* ANTIDIARRHEAL ACTIVITY OF ONCOBA SPINOSA ROOTS

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ABSTRACT

The different extracts of *Oncoba spinosa* (*Flacourtiaceae*) were screened for *in vitro* antidiabetic activity. Of all the extracts tested, ethanolic extract showed highest α-amylase inhibition ranging from 8.64+0.66% to 79.94+0.65% and α-glucosidase inhibition ranging from 14.12+0.51% to 78.68+0.36% when studied at concentrations 62.5-1000µg/mL. The ethanolic extract was subjected to antidiarrheal activity and diarrheal severity was reduced significantly by 15.81% in 100mg/kg group, 30.45% in 200mg/kg group and 74.37% in 400mg/kg group in castor oil induced diarrhea model. In castor oil induced enteropooling, the extract at doses 200mg/kg and 400mg/kg showed 33.46% and 42.44% inhibition of intestinal accumulation. In the charcoal meal test, the distance travelled by charcoal meal was significantly reduced by the extract at doses 200mg/kg and 400mg/kg ($P<0.01$). The overall results tend to suggest the antidiabetic and antidiarrheal activities of *Oncoba spinosa.*

Key word: *In vivo*, *in vitro*, antidiabetic, antidiarrheal, *Oncoba spinosa*, roots

INTRODUCTION

Diabetes mellitus is a group of heterogenous disorders characterised by chronic hyperglycaemia mainly due to absolute (type 1 insulin-dependent) or relative (type 2 noninsulin-dependent) deficiency of insulin hormone. By 2030, the worldwide estimation of diabetic patients is likely to be more than double from that of 2005 without any intervention (Gershell 2005). Every year, about 5% of the deaths worldwide are likely related to diabetes (Hiremath *et al.,* 2010). Diarrheal diseases are one of the leading causes of morbidity and mortality in developing countries and are responsible for the death of millions of people every year (Carlos *et al.,* 1990). Despite immense technological advancement in modern medicine, many people in the developing countries still rely on the healing practices and medicinal plants for their daily health care needs (Ojewole 2008). Therefore, the World Health Organization encouraged studies for the treatment and prevention of diarrheal diseases and diabetes treatment depending on traditional medical practices (Atta *et al.,* 2004).

Oncoba spinosa, the snuff-box tree, fried egg tree or fried-egg flower, is a spiny shrub that belongs to *Flacourtiaceae* which has 95 genera and 800-1000 species. The leaves are dark, glossy green in colour and somewhat leathery and hairless. The fruits are grown up to 60 mm in diameter, it consists of hard shell that becomes dark-reddish brown when mature with shiny seeds embedded in a dry yellowish pulp (Burkil 1984). Fruits and leaves are used for colds, fever, female infertility; seed oil as febrifuge. Traditionally the plant is used in the treatment of diabetes and tumour. The roots of *Oncoba spinosa* are used in the treatment of dysentery and bladder complaints (Burkil 1994). To the best of our knowledge, no reports were available on the antidiarrheal activity and antidiabetic activity of *Oncoba spinosa* roots. The aim of the present work was to study and evaluate the biological activity of roots of *Oncoba spinosa* as an α-glucosidase inhibitor, an α-amylase inhibitor, and an antidiarrheal.

MATERIALS AND METHOD

Collection and identification of the plant materials

Fresh roots of *Oncoba spinosa* were collected from Tirupathi, Andhrapradhesh. The plant was identified and authenticated taxonomically by Assistant professor K. Madhava chetty of the Department of Botany, S.V.University, Tirupathi, Andhra Pradesh, India. A voucher specimen of the collected sample was deposited in the herbarium of the institution for future reference.

Preparation of the extract

The roots are shade dried, made into coarse powder and extraction was done by using cold maceration process for 72h successively by using petroleum ether, chloroform, ethylacetate and ethanol and filtered to get extracts of respective solvents. All the extracts were vaccum dried to obtain chloroform extract (CEOS), ethylacetate extract (EAOS) and ethanol extract (EEOS) respectively. The ethanolic extract was suspended in 1% CMC and used for in-vivo antidiarrheal evaluation.

Experimental animals

Albino wistar rats (weighing between 150-200g) of both sexes were selected for the antidiarrheal studies. They had free access to food and water and were maintained under standard laboratory conditions which included 12h light-dark cycle and temperature of 28-30 degrees centigrade. Animals are allowed for a one week of acclimatization period prior to the study. The experimental protocol was approved by the IAEC (institutional animal ethical committee) and care of the experimental animals was taken according to the CPCSEA guidelines.

In-vitro α-amylase inhibition assay

One hundred micro liter of the test extract was allowed to react with 200µL of α-amylase enzyme and 100µL of 2mM of phosphate buffer (pH-6.9). After 20min incubation, 100µL of 1% starch solution was added. The same was performed for the controls where 100µL of the test extract was replaced by buffer. After incubation for 5min, 500µL of dinitrosalicylic acid reagent was added to both control and test. They were kept in boiling water bath for 5 min. The absorbance was recorded at 540nm using spectrophotometer and the percentage inhibition of α-amylase enzyme was calculated using the below formula. Acarbose was used as positive control.

$$\text{Inhibition (\%)} = 100 \left(\frac{\text{Control} - \text{Test}}{\text{Control}} \right)$$

Suitable reagent blank and inhibitor controls were simultaneously carried out.

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) were determined graphically (Bernfeld, 1955).

In-vitro α-glucosidase assay

A volume of 60µL of sample solution and 50µL of 0.1M phosphate buffer (pH 6.8) containing α-glucosidase solution (0.2U/mL) was incubated at 37ºC for 20min. After pre-incubation, 50µL of 5mM *p*-nitrophenyl-α-D-glucopyranoside (PNPG) solution in 0.1M phosphate buffer (pH 6.8) was added and incubated at 37ºC for another 20min. Then the reaction was stopped by adding 160µL of 0.2M Na_2CO_3, and absorbance readings were recorded at 405nm and compared to a control which had 60µL of buffer solution in place of the extract. For blank incubation (to allow for absorbance produced by the extract), enzyme solution was replaced by buffer solution and absorbance recorded. The α-glucosidase inhibitory activity was expressed as inhibition %. Acarbose was used as positive control (Kaskoos 2013).

$$\text{Inhibition (\%)} = 100 \left(\frac{\text{Control} - \text{Test}}{\text{Control}} \right)$$

Concentrations of extracts resulting in 50% inhibition of enzyme activity (IC_{50}) were determined graphically.

Castor oil induced diarrhea

Rats selected for the experiment are fasted for 18h with only access to water are divided into five groups of six animals each. Group I received vehicle 1% CMC, group II received 100mg/kg of EEOS orally, group III received 200mg/kg of EEOS orally, group IV received 400mg/kg of EEOS orally and group V received atropine 3mg/kg i.p. After 1h of treatment with the extract, standard drug and vehicle, 1mL of castor oil was given orally to all

the five groups of animals. The rats were then placed in metabolic cages and the floor of the cages was lined with adsorbent papers in order to collect the faeces. All the animals are to be observed for four hours for the presence of diarrhea (Shoba *et al.,* 2001; Ujjin *et al.,* 2005).

Antidiarrheal activity of the drug or extract was expressed in terms of percent inhibition of diarrhea. The percent inhibition of defecation was calculated by using the formula:

$$\% \text{ Inhibition of defecation} = \left[\frac{(X-Y)}{X} \right] x100$$

X is the mean number of defecation caused by castor oil and Y is the mean number of defecation caused by drug or extract.

Castor oil induced enteropooling

Rats selected for the experiment are fasted for 18 hours with only access to water are divided into five groups of six animals each. Group I received vehicle 1% CMC, group II received 100mg/kg of EEOS orally, group III received 200mg/kg of EEOS orally, group IV received 400mg/kg of EEOS orally and group V received atropine 3mg/kg i.p. After 1 hour of treatment with the extract, standard drug and vehicle, 1mL of castor oil was given orally to all the five groups of animals. After 1h of treatment with castor oil, rats from all the groups were sacrificed and whole intestine from the pylorus to caecum was removed after tying the ends with thread and weighed. The contents of the intestine were collected by milking into a graduated tube. The intestine was reweighed and the difference between the full and empty intestine was measured in grams (Robert *et al*., 1976).

$$\text{Percentage of intestinal fluid inhibition} = \frac{(T_c - T_t)}{T_c} x100$$

T_c :Mean fluid accumulation in control group and T_t:Mean fluid accumulation in test group

Charcoal meal test or gastrointestinal motility test

Rats selected for the experiment are fasted for 18h with only access to water are divided into five groups of six animals each. Group I received vehicle 1% CMC, group II received 100mg/kg of EEOS orally, group III received 200mg/kg of EEOS orally, group IV received 400mg/kg of EEOS orally and group V received atropine 3mg/kg i.p. After 1h of treatment with the extract, standard drug and vehicle, 1mL of castor oil was given orally to all the five groups of animals. After 1h of treatment with castor oil, 1mL of marker (10% charcoal suspension in 5% gum acacia) was administered orally to all the animals in all the five groups. The rats were sacrificed after one hour and the small intestine was removed surgically and the distance travelled by the charcoal meal from pylorus to caecum was measured after keeping the intestine on a clean transparent clean glass and the values are expressed as percentage with respect to the total length of the intestine from pylorus to caecum (Mascolo *et al.,* 1994). The percentage of inhibition was also calculated by using the following formula (Mujumdar 1998).

$$\text{Distance travelled (\%)} = \frac{\text{Distance travelled by charcoal}}{\text{Total length}} x\ 100$$

$$\text{Inhibition (\%)} = \frac{\text{Total length-Distance travelled by the charcoal}}{\text{Total length}} x\ 100$$

Statistical analysis

Results are expressed as mean±SEM. Data obtained was analyzed by using one way ANOVA followed by Dunnett's test and $p<0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis

Results obtained for qualitative screening of phytochemicals in roots of *Oncoba spinosa*are presented in table I.

In-vitro α-amylase inhibition assay

The α-amylase inhibitory effect of CEOS was found to be ranging from 3.61±0.42% to 26.80±0.58%. The α-amylase inhibitory effect of EAOS was found to be ranging from 5.91+0.56% to 61.18+0.17% when studied at concentrations 62.5-1000µg/mL. At same concentration, the inhibitory effect of EEOS was found to be ranging from 8.64+0.66% to 79.94+0.65% whereas the effect of the standard drug acarbose, ranged from 18.54+0.31% to 82.73+0.65%.

Table I. Qualitative phytochemical analysis of *O. spinosa* roots

S.No	Chemical Constituent	CEOS	EAOS	EEOS
1	Alkaloids	-	-	-
2	Carbohydrates	-	+	+
3	Glycosides	-	+	+
4	Anthroquinones	-	-	-
5	Cardiac glycosides	-	-	-
6	Saponins	-	-	+
7	Steroids	+	-	-
8	Phenols	-	+	+
9	Tannins	-	+	+
10	Proteins	-	+	+
11	Terpenoids	-	-	-
12	Flavonoids	-	+	+

Note: + ve indicates presence, whereas – ve indicates absence. CEOS: Chloroform extract of *Oncoba spinosa,* EAOS: ethyl acetate extract of *Oncoba Spinosa and* EEOS: ethanol extract of *Oncoba spinosa*

Table II. *In vitro* α-amylase inhibitory activity of *Oncoba spinosa* root extracts

Test Substance	% Inhibition Concentration (µg/mL)					IC_{50}(µg/mL)
	62.5µg/mL	125µg/mL	250µg/mL	500µg/mL	1000µg/mL	
CEOS	3.61±0.42	8.07±0.62	12.81±0.46	16.88±0.48	26.80±0.58	>1000µg/mL
EAOS	5.91±0.56	16.36±0.36	31.04±0.57	47.55±0.20	61.18±0.17	589.32µg/mL
EEOS	8.64±0.66	20.72±1.15	41.43±0.99	69.90±0.47	79.94±0.65	308.19µg/mL
Acarbose (Standard)	18.54±0.31	29.30±0.25	48.05±0.67	72.61±0.25	82.73±0.65	246.90µg/mL

CEOS=chloroform extract of *Oncoba spinosa;* EAOS=ethylacetate extract of *Oncoba spinosa,* EEOS=ethanol extract of *Oncoba Spinosa.* Values are expressed as mean±SEM of triplicate measurement

The IC_{50} value of ethylacetate extract, EAOS was found to be 589.32µg/mL whereas the ethanolic extract, EEOS showed at 308.19µg/mL. The IC_{50} of acarbose was found to be 246.90µg/mL (Table II).

In vitro α-glucosidase inhibition assay

The α-glucosidase inhibitory effect of CEOS was found to be ranging from 2.98±0.26% to 29.29±0.44%. The α-glucosidase inhibitory effect of EAOS was found to be ranging from 6.43+0.01% to 55.21+0.28% when studied at concentrations 62.5-1000µg/mL. At same concentration, the inhibitory effect of EEOS was found to be ranging from 14.12+0.51% to 78.68+0.36% whereas the effect of the standard drug acarbose, ranged from 19.92+0.39 to 82.50+0.39%. The IC_{50} value of ethylacetate extract, EAOS was found to be 914.98µg/mL whereas the ethanolic extract, EEOS showed at 300.39µg/mL. The IC_{50} of acarbose was found to be 251.89µg/mL (Table III).

Castor oil-induced diarrhea

In castor-oil induced diarrhea, EEOS was found to be effective in a dose dependent manner against castor oil induced diarrhea. The effect of EEOS at the dose levels of 100, 200 and 400 mg/kg caused a dose dependent decrease in the number of faecal matter from 25.33, 18.16 and 8.16 respectively. However, percent reduction in total number of faecal matter ranges from 16.95 to 73.24%. EEOS at 100 mg/kg showed 15.81% inhibition of diarrhea whereas doses 200mg/kg and 400 mg/kg showed 30.45% and 74.37% inhibition respectively. The standard drug atropine showed 80.52% inhibition of diarrhea. The results are shown in table IV.

Table III. *In vitro* α-glucosidase inhibitory activity of *Oncoba spinosa* root extracts

Test Substance	%Inhibition Concentration (µg/mL)					IC50 (µg/mL)
	62.5µg/mL	125µg/mL	250µg/mL	500 µg/mL	1000 µg/mL	
CEOS	2.98±0.26	5.94±0.44	13.97±0.32	22.63±0.08	29.29±0.44	>1000µg/mL
EAOS	6.43±0.01	11.98±0.48	21.79±0.23	37.03±0.12	55.21±0.28	914.98µg/mL
EEOS	14.12±0.51	26.68±0.49	41.79±0.13	66.09±0.32	78.68±0.36	300.39µg/mL
Acarbose (Standard)	19.92±0.39	33.23±0.28	46.40±0.53	66.70±0.32	82.50±0.39	251.89µg/mL

CEOS=chloroform extract of *Oncoba spinosa*; EAOS=ethylacetate extract of *Oncoba spinosa*, EEOS=ethanol extract of *Oncoba spinosa*. Values are expressed as mean±SEM of triplicate measurement

Table IV. Effect of EEOS on castor oil induced diarrheal

Treatment Group	Total number of faeces	%inhibition of faeces	Total number of diarrheal faeces	%inhibition of diarrhea
Vehicle plus castor oil 1mL	30.50±2.14	-	13.66±0.84	-
EEOS-100mg/kg	25.33±1.94	16.95	11.50±0.42*	15.81
EEOS-200mg/kg	18.16±1.30**	40.45	9.50±0.61**	30.45
EEOS-400mg/kg	8.16±0.54**	73.24	3.50±0.42**	74.37
Atropine-3 mg/kg	4.66±0.61**	84.72	2.66±0.33**	80.52

Values are expressed as mean±SEM (n=6 in each group). *P<0.05 and **P<0.01 when compared to castor oil control group.

Table V: Effect of EEOS on castor oil induced enteropooling

Treatment Group	Weight of the intestinal content (g)	Volume of intestinal fluid (mL)	% inhibition
Vehicle plus castor oil 1mL	6.10±0.58	2.45±0.33	-
EEOS-100mg/kg	5.93±0.16^{ns}	2.23±0.16^{ns}	8.97
EEOS-200mg/kg	4.48±0.33*	1.63±0.20*	33.46
EEOS-400mg/kg	2.33±0.26**	1.41±0.13**	42.44
Atropine-3mg/kg	1.50±0.10**	0.71 ±0.13**	71.02

Values are expressed as mean±SEM (n=6 in each group). *P<0.05 and **P<0.01 when compared to castor oil control group.

Castor oil-induced enteropooling

EEOS showed a significant reduction in castor oil-induced fluid accumulation at 200mg/kg and 400mg/kg with respective values of 1.63±0.20 ($p<0.05$) and 1.41±0.13 ($p<0.01$). Atropine significantly reduced ($p<0.01$) the fluid accumulation with value of 0.71±0.13 at the dose of 3mg/kg. EEOS reduced the castor oil induced intraluminal accumulation of fluid by 8.97, 33.46 and 42.44% at doses of 100, 200 and 400mg/kg respectively. Results are shown in table V.

Charcoal meal test

In charcoal meal test, the extract at 100mg/kg dose did not show any significant reduction in the distance travelled by the charcoal meal when compared to the castor oil control group. The gastrointestinal distance travelled by the charcoal meal in the rats significantly reduced by the extract at the doses 200mg/kg and 400mg/kg ($P<0.01$). In charcoal meal test, the percent inhibition of charcoal meal was found to be 8.94±1.23 in control group, 13.26±2.51 in 100mg/kg dose group,

Table VI: Effect of EEOS on charcoal meal test

Treatment Group	Length of the intestine (cm)	Distance travelled by the charcoal meal (cms)	Distance travelled by the charcoal meal (%)	% Inhibition
Vehicle plus castor oil 1mL	72.83±1.60	61.00 ±1.71	89.04 ±1.33	8.94 ±1.23
EEOS-100mg/kg	63.33±1.43	56.00 ±1.12^{ns}	83.72±1.11	13.26 ±2.51 ^{ns}
EEOS-200mg/kg	68.66±1.10	35.00±1.50**	51.17 ±2.12**	47.81 ±2.42**
EEOS-400mg/kg	65.66±2.46	23.00 ±1.25**	38.17 ±2.51**	53.61 ±2.31**
Atropine-3mg/kg	69.12±2.12	16.00±1.32**	23.12 ±1.52**	73.16 ±1.52**

Values are expressed as mean±SEM (n=6 in each group). **P<0.01 when compared to castor oil control group

47.81±2.42 in 200mg/kg dose group (p<0.01), 53.61±2.31 in 400 mg/kg dose group (p<0.01) and 73.16±1.52 in standard group (p<0.01). Results are shown in table VI.

Hyperglycemia has been a classical risk in the development of diabetes and the complications associated with diabetes. Therefore control of blood glucose levels is critical in the early treatment of diabetes mellitus and reduction of macro- and microvascular complications. One therapeutic approach is the prevention of carbohydrate absorption after food intake, which is facilitated by inhibition of enteric enzymes including α-glucosidase and α-amylase present in the brush borders of intestine. In this study, the α-amylase and α-glucosidase inhibitory activity of the roots of *Oncoba spinosa* was investigated. The inhibitory effect of CEOS, EAOS, and EEOS were analysed. Alpha amylase converts starch to maltose which reduces 3,5-dinitrosalicylic acid to 3-amino-5-nitrosalicylic acid. Inhibition of α-amylase resulted in a decreased production of maltose. This maltose will reduce the 3,5-dinitrosalicylic acid in the colouring agent to 3-amino-5-nitrosalicylic acid. This reaction will produce a colour change from orange to red.

Starch + H_2O+ α-amylase -->Maltose (red colour)

The intensity of red colour will be directly proportional to the amount of maltose produced. When an enzyme inhibitor is present in the reaction mixture, digestion of starch, production of maltose and intensity of red colour produced will be less (Rao *et al*., 2008). α--glucosidase will catalyze the conversion of the substrate 4-nitrophenyl-D-glucopyranoside to D-glucopyranoside and p-nitrophenol. The yellow colour of the latter product is measured spectrophotometrically at 405 nm.

PNPG + α-glucosidase --> α-D-glucopyranoside + PNP (yellow colour)

The intensity of the colour produced is directly proportional to extent of digestion of PNPG and released p-nitro phenol. Thus in the presence of an inhibitor, digestion of PNPG, production of p-nitro phenol and intensity of colour produced will be less (Subramanian *et al.,* 2008). Acarbose, a known α-amylase and α-glucosidase inhibitor, currently used in antidiabetic therapy for reducing postprandial increase in blood glucose levels, reduced the intensity of the colour produced. The plant extracts (EAOS and EEOS) also produced decrease in colour intensities in both the in vitro assays suggesting that they also possess inhibitory potential against α-amylase and α-glucosidase enzymes. Previous reports suggest that various potential inhibitors isolated from medicinal plants belong to flavonoid and glycoside class (Lee *et al*., 2008; Jong *et al.,* 2007). Based on the preliminary phytochemical analysis, we speculate that the presence of flavonoids and glycosides might have contributed to the α-amylase and α-glucosidase inhibitory activity.

The ethanolic extract which showed highest α-amylase and α-glucosidase inhibition was selected for antidiarrheal activity. Castor oil increases volume of intestinal content by prevention of the re-absorption of water and the liberation of ricinoleic acid from castor oil

results in irritation and inflammation of the intestinal mucosa leading to release of prostaglandins which results in stimulation of motility and secretion and the prevention of re-absorption of NaCl and water (Das *et al*., 2007). This is characterized by an increase in the secretion of water and electrolytes, an increase in intestinal transit time and an increase in wet faeces (Oben *et al.,* 2006). Our results showed that the extract inhibited significantly castor oil induced diarrhea in rats and the effect was almost similar to standard drug. The plant extract may have brought about this activity by re-absorption of water from the intestinal lumen or by anti prostaglandin activities that contribute to patho-physiological functions in the gastrointestinal tract. The results of our present study showed the extract at the doses 200 mg/kg ($p<0.05$) and 400 mg/kg ($p<0.01$) significantly reduced the volume of intestinal fluid accumulation in rats. The prevention of intraluminal fluid secretion by *Oncoba spinosa* in this study may be due to inhibition of prostaglandin biosynthesis with resultant decrease in secretion of fluid into the lumen or may be due to promotion of absorption of water and electrolytes in the gut. In charcoal meal test, administration of EEOS in rats caused a significant reduction in the progression of charcoal meal and in the intestinal transit time. This activity is comparable to that of atropine used here as a reference drug and which is known to reduce the intestinal motility (longanga *et al.,* 2000). The antidiarrheal effect of EEOS could thus result from a reduction of intestinal motility and an increase in the intestinal absorption of water and electrolytes. In fact, many previous studies have shown that drugs and natural products as well, can induce their antidiarrheal effect through antispasmodic activity (Rehman *et al.,* 2009).

Preliminary phytochemical analysis of ethanolic extract showed the presence of carbohydrates, glycosides, saponins, flavonoids, phenols, tannins and proteins. Previously various medicinal plants were screened for antidiarrheal activities and the activity is due to presence of tannins, alkaloids, saponins, flavonoids, steroids, terpenes and glycosides (Longanga *et al.,* 2000). The presence of some of these phytochemical constituents in *Oncoba spinosa* may be responsible for the antidiarrheal effect.

CONCLUSION

In conclusion, the results of this investigation revealed that ethanolic extract of *Oncoba spinosa* contains pharmacologically active substances with antidiarrheal and antidiabetic properties. These attributes may provide the rationale for the use of *Oncoba spinosa* in traditional medicine for the treatment of diarrhea and diabetes. Further research is needed to fractionate the ethanolic extract and isolate the molecule(s) responsible for the antidiarrheal and antidiabetic activity.

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